Inhibition of Morphine-Induced Pituitary–Adrenal Activation by Dexamethasone in the Female Rat[1] (37506)

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It is well established that morphine sulfate (MS) markedly stimulates pituitary–adrenal function in the unanesthetized rat (1, 2). Recently, Kokka *et al.* (3) reported complete inhibition of this response to MS by prior administration of a large dose (600 μg/kg) of the synthetic corticosteroid, dexamethasone (Dex). The present study was performed to examine the sensitivity of MS-induced pituitary–adrenal activation to the inhibitory action of relatively low doses of Dex in the female rat.

Materials and Methods. Adult female rats (Charles-River, CD), weighing 250–300 g, were used. All animals were from the same shipment and were housed 3/cage for 3 wk under conditions of controlled lighting (fluorescent illumination from 04:00–18:00 alternating with darkness from 18:00–04:00) and temperature (26 ± 2°). Purina Lab Chow and tap water were available at all times. Three days prior to the experiment, the rats were transferred to individual cages. At noon on the day of the experiment, 8 rats received 5 μg/kg, 8 rats received 20 μg/kg, and 16 rats received 100 μg/kg Dex[2] sc (approx 0.5 ml), freshly dissolved in normal saline; controls received saline sc. Two hours later, 8 rats from each treatment group received ip 30 mg/kg MS dissolved in saline and the remaining 8 Dex-treated and 8 saline-treated rats were given saline ip (approx 0.5 ml). Two hours later, *i.e.*, at 16:00, rats were taken individually to a laboratory adjoining the animal room and experimental procedures were begun within 15 sec following cage opening. These procedures involved 3-min exposure to ether vapor during which an external jugular vein was exposed and an initial 1.5 ml sample of blood was withdrawn into a heparinized syringe for determination of the prestress level of corticosterone in plasma. Previous studies (4) demonstrated that corticosterone levels in plasma obtained within 3 min following onset of stress (cage opening) are similar to those obtained by rapid decapitation immediately following cage opening. Each rat was reetherized at 13 min and a second 1.5 ml sample of jugular blood was obtained at 15 min for determination of the stress plasma level of corticosterone. The difference between prestress and stress levels of corticosterone was calculated for individual rats and used as an index of the pituitary–adrenal response to stress. Blood samples were centrifuged and the plasma was separated and frozen for subsequent fluorometric determination of corticosterone concentrations (5). No correction for residual fluorescence was made; however, plasma from adrenalectomized female rats in this laboratory contains the equivalent of approximately 6 μg corticosterone/100 ml.

The experiment was performed according to a randomized block design. Statistical probabilities were determined by analysis of variance and Duncan's Multiple Range Test (6) performed by the Common Research Computer Facility[3] with the program Exbiol (7).

Results. The results are shown in Fig. 1. Controls treated with saline at 12:00 and 14:00 showed prestress levels of plasma corticosterone that are typical of the late afternoon diurnal peak (8). Consistent with re-

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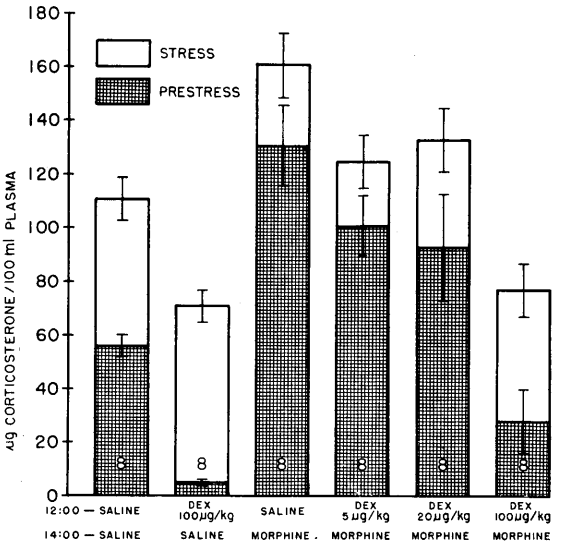

FIG. 1. Effects of 30 mg/kg morphine (prestress) and 3-min ether (stress) on plasma corticosterone levels following pretreatment with various doses of dexamethasone or saline in the female rat. Figures in columns denote numbr of rats and vertical lines represent ± standard error.

sults of previous studies (9), 100 μg/kg Dex caused complete suppression of diurnal peak in prestress levels of corticosterone. Compared to controls that received saline at both 12:00 and 14:00, MS clearly caused elevation of prestress corticosterone concentrations when administered following saline ($p < 0.01$). This elevation in prestress steroid levels produced by MS was significantly suppressed by prior administration of 20 ($p = 0.05$), but not by 5 ($p > 0.05$) μg/kg Dex, and it was markedly suppressed ($p < 0.01$) by pretreatment with 100 μg/kg Dex. The prestress steroid levels in rats that received the largest dose of Dex (100 μg/kg) before MS were somewhat but not significantly higher than those of rats treated with Dex and saline.

Exposure to 3-min ether stress resulted in significant corticosterone increments in all groups. However, there were some differences in amplitude of responses to ether; steroid increments in rats treated with saline plus MS or with 5 μg/kg Dex plus MS were smaller ($p < 0.05$) than that in the saline–saline controls. The steroid responses in rats treated with 100 μg/kg Dex plus saline and those that received 20 or 100 μg/kg Dex prior to MS were comparable to that of the saline–saline controls.

Discussion. These results demonstrate dose-dependent inhibition by Dex of MS-induced activation of the pituitary–adrenal axis. In the absence of Dex, plasma corticosterone levels were markedly elevated 2 hr after injection of 30 mg/kg MS. This conspicuous elevation in corticosterone levels was not significantly suppressed by prior treatment with 5 μg/kg of Dex, but it was partially suppressed by 20 μg/kg and almost completely inhibited by 100 μg/kg of this synthetic steroid. The apparent susceptibility of MS-induced pituitary–adrenal activation to feedback inhibition by glucocorticoids is consistent with the findings of others. Thus, Kokka *et al.* (3) observed total suppression of plasma corticosterone levels 30 min after injection of either 20 or 40 mg/kg MS in rats pretreated with 600 μg/kg of Dex. In addition, George and Way (10) reported that prior administration of 5 or 10 mg/kg of cortisone reduced the adrenal ascorbic acid-depleting effects of 30 mg/kg MS in the rat. The present results extend these previous findings by demonstrating that the corticosteroid response to MS is sensitive to the

feedback effects of a relatively low dose of Dex. Furthermore, it seems that this sensitivity is roughly comparable to that of nonstress pituitary–adrenal function (9) and of responses to certain stress stimuli (11). Therefore, MS appears to be another potent yet relatively corticosteroid-sensitive stimulator of the pituitary–adrenal system in the rat. The persistence of responses to 3-min exposure to ether in all groups in the present studies is consistent with previous observations which indicate that this particular response is refractory to the doses of Dex used (9). The limited corticosterone response to ether stress in groups that received MS alone or in combination with less than 100 μg/kg Dex probably represents a ceiling effect determined by maximum adrenal secretory capacity.

Blockade of MS-induced activation of the pituitary–adrenal system in the rat has also been achieved by prior administration of various central nervous system depressants (13) and with the specific narcotic antagonist, nalorphine (10, 13). George and Way (10) found that 5 mg/kg cortisone was as effective as 5 mg/kg nalorphine in suppressing the adrenal ascorbic acid depletion caused by 30 mg/kg MS in the rat. By comparison, the nearly complete suppression of the corticosterone response to MS obtained in the present study with 100 μg/kg Dex indicates that this synthetic steroid is a potent antagonist of the presumed central action of MS that results in ACTH release.

The site at which MS acts to stimulate the pituitary–adrenal axis and the site of blockade by Dex are unknown. Because Lotti, Kokka and George (12) reported increased levels of corticosterone in rats following infusion of MS into the hypothalamus, this drug may act at the hypothalamus and/or pituitary. Results from several approaches (14) indicate that Dex also affects ACTH secretion via hypothalamus and/or pituitary. Consistent with these presumptive sites of action for both agents, unpublished data in this laboratory suggest that MS-induced stimulation of ACTH secretion and its blockade by systemic Dex persist in rats subjected to complete surgical isolation of the medial basal hypothalamus.

Summary. Adult female rats received 30 mg/kg morphine sulfate (MS) ip 2 hr after sc injection of various doses of dexamethasone (Dex) or saline. Plasma corticosterone levels were markedly elevated 2 hr after MS and such elevation was partially suppressed by pretreatment with 20 μg/kg Dex and markedly inhibited by 100 μg/kg of this steroid; 5 μg/kg failed to cause significant suppression of plasma corticosterone levels. Despite treatment with Dex and MS, all groups showed significant corticosterone responses to 3-min ether stress. Because of the relatively low doses used, Dex appears to be a potent inhibitor of the ACTH-releasing action of MS.

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